

# Preparation of uniform rich cholesterol unilamellar nanovesicles using CO<sub>2</sub>-expanded solvents

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## Supporting Information

### Solubility measurements

Solubility measurements of cholesterol in CO<sub>2</sub>-expanded acetone were performed in a home made high pressure phase equilibrium analyzer. The main components of this configuration are a Jergusson T-40 view cell, a JASCO PU-1580 HPLC pump to introduce liquid solvents or solutions, an ISCO 260D pump to introduce CO<sub>2</sub> and a micropump to re-circulate the internal mixture. The variation of the cell volume is

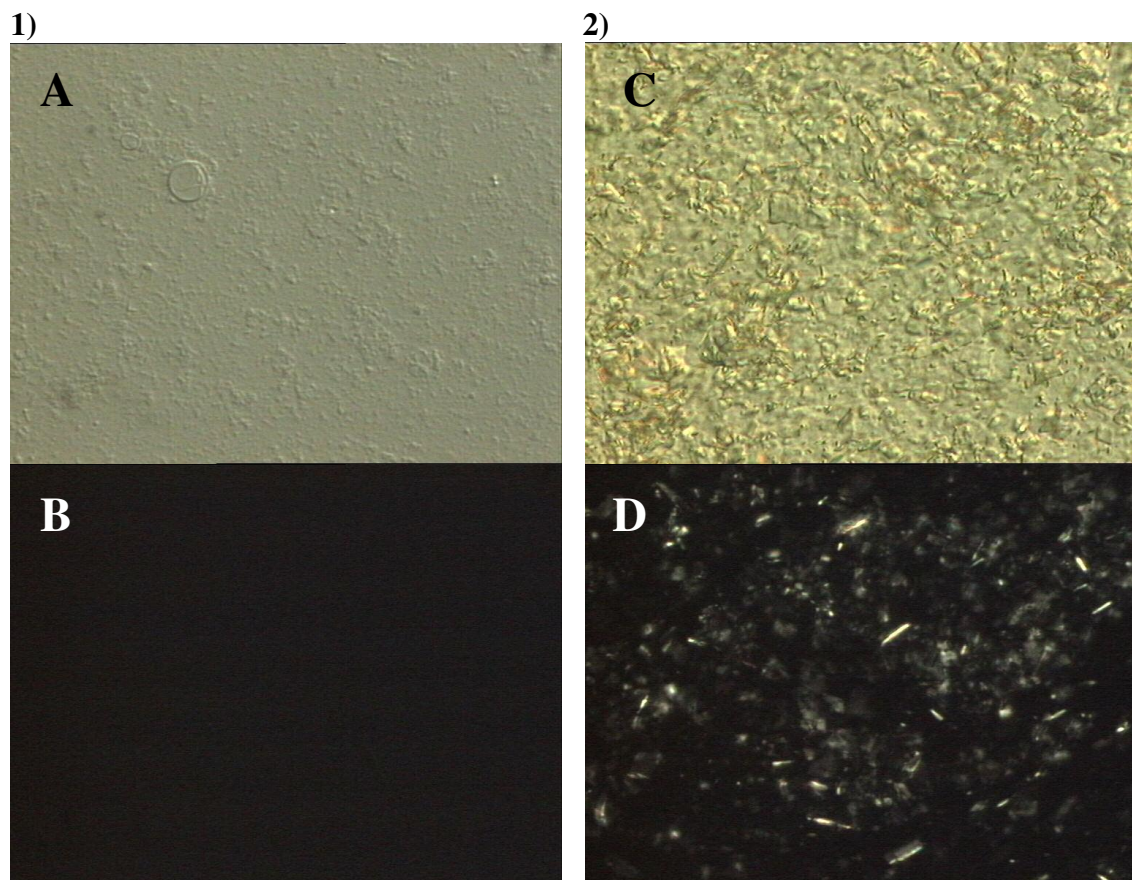
performed by means of a stainless-steel piston introduced into the view cell and moved by a pneumatic system. In this way, the cell internal volume can be varied from 50 to 75mL. Solid samples can be introduced into the cell previously to pressurization by a stainless steel cylinder supported in a Teflon base. The vanishing-point method was employed to measure solubility of cholesterol at 10 MPa, 313K and at different solvent composition,  $X_{CO_2}$ . The use of this procedure with our equipment has a systematic relative error lower than 1% in the determination of solubilities and lower than 2% in the determination of  $CO_2$  molar fractions. These errors arise from the weighing of the solute and organic solvent components, from the  $CO_2$  volume delivery and from the calculation of  $CO_2$ -density in the pump. Solubilities in acetone and  $CO_2$  neat solvents were measured through a static and a dynamic method respectively. In both methods the systematic relative errors were lower than 1%.

**Solubilities of cholesterol in different “acetone- $CO_2$ ” expanded solutions at**

**$P_w = 10$  MPa and  $T_w = 308$  K, measured through the “Vanishing Point” method**

$X_{CO_2}$	$C_s$ (mol cholesterol/mol solvent)
0	6.42E-03
0.082	5.75E-03
0.222	4.52E-03
0.352	3.84E-03
0.458	3.27E-03
0.569	2.41E-03
0.657	1.72E-03
0.704	1.08E-03
0.798	6.26E-04
0.9	2.12E-04
1	5.00E-05

**Optical microscope images of cholesterol dispersed systems obtained by DELOS-SUSP procedure in the presence and absence of cetyl trimethyl ammonium bromide (CTAB)**



1) Optical microscope images of the system “cholesterol/CTAB/water/acetone” obtained by a DELOS-SUSP procedure, viewed by (A) transmission and (B) under polarized light.

2) Optical microscope images of the system “cholesterol/water/acetone” in the absence of CTAB surfactant a DELOS-SUSP procedure, viewed by (C) transmission and (D) under polarized light.

Both systems were obtained using the same DELOS-SUSP operational parameters, and keeping constant the molar relationship between cholesterol, water and acetone.